Isolation, Identification and Antimicrobial Susceptibility pattern of *Pseudomonas Aeruginosa* from Various Clinical Specimens at a North India Hospital.

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ABSTRACT

Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram negative bacilli. It is an opportunistic human pathogen and plays an important role in nosocomial infection. It is hard to treat because some factor and several mechanisms are involved in resistant organism. Aim: Isolation, Identification and Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*. **Methods:** This study was conducted during October 2014 to September 2015.Total 2492 samples were collected in which 822 samples show growth. Out of 822, 68 samples were positive for Pseudomonas aeruginosa on the basis of their growth on culture media, oxidase test and biochemical tests. The Antimicrobial susceptibility test of isolates was performed by Kirby-Bauer disc diffusion method according to CLSI guidelines (2014). **Results:** Majority of *Pseudomonas aeruginosa* was isolated from pus, urine and swab. The isolated pathogens were maximum sensitivity to Imepenem (88.24%) followed by Meropenem (83.82%), Piperacillin-Tazobactam (82.35%) and were maximum resistance to Gentamicin (61.76%), Tobramycin (60.29%).**Conclusion:** To conclude, Imepenem, Meropenem and Piperacillin-Tazobactam were found to be the most effective antimicrobial drugs. It should be used in limit. The use of Gentamicin and Tobramycin should be reduced.

Keywords: *Pseudomonas aeruginosa*, Opportunistic, Nosocomoal infection, Antimicrobial sensitivity.

INTRODUCTION

Pseudomonas aeruginosa is a non-fermenting, gram negative aerobic bacilli, motile, and its family is Pseudomonadaceae.[1-4]

It is a ubiquitous and opportunistic human pathogen, usually founds in humans, animals, plants, soil, water as well as the moist environment in hospitals and related to high morbidity and mortality as well as healthcare cost in hospitals and in community.[5,6] High morbidity and mortality rate mostly seen in patients admitted in Surgery wards, Intensive care unit (ICU) and in Burn unit.[7]

Generally *Pseudomonas aeruginosa* spreads person to person rarely.[8] It can be transmitted by hospital equipment such as catheters, respiratory care equipments and by diluting antiseptics, cleaning liquids, irrigating solutions.[9-11]

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Pseudomonas aeruginosa is an opportunistic human pathogen, and associated with a wide range of nosocomial infection like nosocomial pneumonia, skin and soft tissue infections and urinary tract infections (UTIs).[12]

Infections of *Pseudomonas aeruginosa* are often life-threatening and its treatment is very difficult because it shows more resistance against many antimicrobial agents which is used and increases the frequency of multidrug resistance (MDR) in healthcare society.[13,14]

Some factors such as intensive use of antibiotics in immunocompromised patients and more use of invasive procedures are responsible for resistance to antibiotic and increased involvement of Pseudomonas aeruginosa in infections.[15]

The resistance of *P.aeruginosa* against antimicrobials is due to several mechanisms such as β- lactamase production, cell wall permeability, modifying enzymes and aminoglycosides.[16]

The current study was designed for isolation, identification and also for antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* from various clinical specimens.

MATERIALS AND METHODS

This study was conducted at the department of Microbiology at Hind Institute of Medical Sciences, Safedabad, Barabanki, Lucknow (India) during October 2014 to September 2015. During these period, total 2492 clinical specimens like Pus, Urine, Blood, CSF, Endotrachial tube, Swab (Ear swab, Throat swab, High vaginal swab, Nasal swab), Sputum, Ascitic fluid, Pleural fluid were collected and transported to the Microbiology

department for culture and antibiotic susceptibility test.

Laboratory Isolation and Identification of Isolates

Clinical specimens were inoculated on routine culture media like Mac-Conkey agar, Blood agar, Nutrient agar and Selective media like Cetrimide agar plates, and incubated at 37^0C for 18-24 hours. After that identification was done on the basis of colony morphology, pyocyanin (blue, green) pigmentation, gram staining, motility test, enzymatic test, and biochemical tests etc. Given in [Table 1].

Antimicrobial susceptibility test

The Antimicrobial susceptibility of *Pseudomonas aeruginosa* was determined on Mueller Hinton agar using the Modified Kirby –Bauer disc diffusion method and result were reported according to CLSI guideline (2014).

Pseudomonas aeruginosa were tested for their susceptibility to the following anti-Pseudomonal antimicrobials: Ceftazidime (30 mcg), Gentamicin (30 mcg), Tobramycin (10 mcg), Piperacillin (100 mcg), Amikacin (30 mcg), Aztreonam (30 mcg), Cefepime (30 mcg), Ciprofloxacin (5 mcg), Levofloxacin (5 mcg), Imepenam (10 mcg), Meropenem (10 mcg), Piperacillin-Tazobactum (100/10 mcg), and Norfloxacin (10 mcg: only used in uro-pathogens).

Table 1: Identification of *Pseudomonas aeruginosa*

S. NO.	Tests		Result
1	Gram staining		Gram negative bacilli
2	Colony Morphology	Nutrient agar	Bluish green coloured colonies
		MacConkey agar	Non- lactose fermenting colonies
		Blood agar	Haemolytic colonies
		Cetrimide agar	Bluish green coloured colonies
3	Motility test	By Hanging drop preparation	Positive (Motile)
4	Biochemical tests	Oxidase	Positive
		Catalase	Positive
		Oxidative on O-F medium	Positive
		Indole	Negative
		Methyl red test	Negative
		Voges-Proskauer test	Negative
		Citrate utilization test	Positive
		Urease test	Negative
		Triple Sugar Iron (TSI) test	Alkaline slant, alkaline butt, No H_2S, and No gas
		Nitrate reduction test	Positive
		Gelatin hydrolysis test	Positive

RESULTS

In our study, total 2492 samples were collected in which 1670 were negative and 822 were positive [Table 2]. Out of total 822 positive samples, 68 (8.27%) *Pseudomonas aeruginosa* were isolated, 38 from males and 30 from females [Table 3] in different age group [Table 4], the maximum number was 26 (38.23%) in the age group of 21-40 and minimum number was 10 (14.70%) in the age group of >60. According to type source, the higher number of *Pseudomonas aeruginosa*, 36 (52.94%) was found in pus [Table 5]. In our finding, The Antimicrobial susceptibility pattern of isolated *Pseudomonas aeruginosa* showed maximum sensitivity to Imepenem (88.24%) followed by Meropenem (83.82%) and Piperacillin-Tazobactam (82.35%) and Gentamicin (61.76%) show maximum resistance.

Table 2: Result and percentage of total sample.

Result of Cultured sample	Number of isolates	Percentage (%) Out of total samples
Total positive samples	822	32.99
Negative	1670	67.01
Total	2492	100

Table 3: Sex wise distribution of isolated *P. aeruginosa*.

Gender	Total No.	Percentage (%)
Male	38	55.89
Female	30	44.11
Total	68	100

Table 4: Age wise distribution of isolated *P. aeruginosa*

Age group (years)	No. of Isolates	Percentage (%)
0 – 20	18	26.48
21 – 40	26	38.23

41 – 60	14	20.59
> 60	10	14.70

Total	68	100

Table 5: Distribution of *Pseudomonas aeruginosa* according to source of isolates.

Type of clinical specimens	Total positive Specimens (n=822)	Positive for Pseudomonas aeruginosa (n=68)	
		Number (N)	Percentage (%)
Pus	169	36	52.94
Urine	514	16	23.53
Swab	26	12	17.65
CSF	3	2	2.94
Endotrachial tube	4	2	2.94
Blood	87	0	0
Others	19	0	0
Total	822	68	100

Table 6: Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*.

Antibiotics	Sensitive		Resistance		Total %
	No.	%	No.	%	
Ceftazidime	51	(75)	17	(25)	100
Gentamicin	26	(38.24)	42	(61.76)	100
Tobramycin	27	(39.71)	41	(60.29)	100
Piperacillin	38	(55.88)	30	(44.12)	100
Amikacin	50	(73.53)	18	(26.47)	100
Aztreonam	43	(63.24)	25	(36.76)	100
Cefepime	54	(79.41)	14	(20.59)	100
Ciprofloxacin	37	(54.41)	31	(45.59)	100
Levofloxacin	49	(72.06)	19	(27.94)	100
Imepenem	60	(88.24)	8	(11.76)	100
Meropenem	57	(83.82)	11	(16.18)	100
Piperacillin-tazobactam	56	(82.35)	12	(17.65)	100
Norfloxacin	8	(50.00)	8	(50.00)	100

DISCUSSION

Total 68 *Pseudomonas aeruginosa* were isolated from various clinical specimens.

In the present study, the age wise prevalence of *P. aeruginosa* from various clinical specimens, the maximum patients were aged between 21 – 40 years (38.23%). The most prevalence of *P. aeruginosa* isolated from this group due to prolonged hospitalization and decreased their immunity. Maximum prevalence of *P. aeruginosa* in male group (55.89%) than female (44.11%) because of the most male patients admitted in the hospital and inserted catheter (urethral catheter) for urination. The prolonged catheterisation is also a source of infection, which causes Hospital acquired infection such as Urinary tract infection.

The most prevalence of *P.aeruginosa* from pus (52.94%), urine (23.53%), and swab (17.65%). The distribution of specimens of *P.aeruginosa* varies from each hospital due to environmental factors.

In our study, Imepenem (88.24%) and Meropenum (83.82%) show most sensitive anti-pseudomonal drugs. This is due to minimum use of these antibiotics in this hospital. Gentamicin (61.76%) and Tobramycin (60.29%) show maximum resistance due to altered target sites, bacterial efflux pumps, loss of membrane protein and hospital *P. aeruginosa* strains, which are multi-drug strains transmitted in the hospital during catheterisation and therapeutic procedure, etc. But our study did not isolate multidrug resistance strains of *P. aeruginosa*.

Isolation, Identification and Antibiotic susceptibility pattern of *P. aeruginosa* was comparable with pervious study.[17,18]

CONCLUSION

Result of our study clarifies that most sensitive Pseudomonal drug is Imepenem and most resistance drugs are Gentamicin and tobramycin. So, we suggest the physician to minimize prescribe the Imepenem.

Pseudomonas aeruginosa transmitted in the hospital during patient admitted in the hospital and their therapeutic procedure. So, proper setting of a hospital is necessary and also a monitoring of antimicrobial drugs yearly, which helps to prevent the drug resistance show by *P. aeruginosa*.

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